Effect of nocturnal oxygen and acetazolamide on exercise performance in patients with pre-capillary pulmonary hypertension and sleep-disturbed breathing: randomized, double-blind, cross-over trial

Ulrich, Silvia ; Keusch, Stephan ; Hildenbrand, Florian F ; Lo Cascio, Christian ; Huber, Lars C ; Tanner, Felix C ; Speich, Rudolf ; Bloch, Konrad E

Abstract: AIM: Sleep-disturbed breathing (SDB) is common in pre-capillary pulmonary hypertension (PH) and impairs daytime performance. In lack of proven effective treatments, we tested whether nocturnal oxygen therapy (NOT) or acetazolamide improve exercise performance and quality of life in patients with pre-capillary PH and SDB. METHODS: This was a randomized, placebo-controlled, double-blind, three period cross-over trial. Participants received NOT (3 L/min), acetazolamide tablets (2 × 250 mg), and sham-NOT/placebo tablets each during 1 week with 1-week washout between treatment periods. Twenty-three patients, 16 with pulmonary arterial PH, 7 with chronic thromboembolic PH, and with SDB defined as mean nocturnal oxygen saturation <90% or oxygen saturation dips >10 h(-1) with daytime PaO2 7.3 kPa participated. Assessments at the end of the treatment periods included a 6 min walk distance (MWD), SF-36 quality of life, polysomnography, and echocardiography. RESULTS: Medians (quartiles) of the 6 MWD after NOT, acetazolamide, and placebo were 480 m (390;528), 440 m (368;468), and 454 m (367;510), respectively, mean differences: NOT vs. placebo +25 m (95% CI 3-46, P= 0.027), acetazolamide vs. placebo -9 m (-34-17, P = 0.223), and NOT vs. acetazolamide +33 (12-45, P < 0.001). SF-36 quality of life was similar with all treatments. Nocturnal oxygen saturation significantly improved with both NOT and acetazolamide. Right ventricular fractional area change was greater on NOT compared with placebo (P = 0.042) and acetazolamide (P = 0.027). CONCLUSIONS: In patients with pre-capillary PH and SDB on optimized pharmacological therapy, NOT improved the 6 MWD compared with placebo already after 1 week along with improvements in SDB and haemodynamics. CLINICALTRIALS.GOV: NTC01427192.

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Effect of nocturnal oxygen and acetazolamide on exercise performance in patients with pre-capillary pulmonary hypertension and sleep-disturbed breathing: randomized, double-blind, cross-over trial

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Aim Sleep-disturbed breathing (SDB) is common in pre-capillary pulmonary hypertension (PH) and impairs daytime performance. In lack of proven effective treatments, we tested whether nocturnal oxygen therapy (NOT) or acetazolamide improve exercise performance and quality of life in patients with pre-capillary PH and SDB.

Methods This was a randomized, placebo-controlled, double-blind, three period cross-over trial. Participants received NOT (3 L/min), acetazolamide tablets (2 × 250 mg), and sham-NOT/placebo tablets each during 1 week with 1-week washout between treatment periods. Twenty-three patients, 16 with pulmonary arterial PH, 7 with chronic thromboembolic PH, and with SDB defined as mean nocturnal oxygen saturation <90% or oxygen saturation dips >10 h^{-1} with daytime $PaO_2 \geq 7.3$ kPa participated. Assessments at the end of the treatment periods included a 6 min walk distance (MWD), SF-36 quality of life, polysomnography, and echocardiography.

Results Medians (quartiles) of the 6 MWD after NOT, acetazolamide, and placebo were 480 m (390;528), 440 m (368;468), and 454 m (367;510), respectively, mean differences: NOT vs. placebo +25 m (95% CI 3–46, $P = 0.027$), acetazolamide vs. placebo −9 m (−34–17, $P = 0.223$), and NOT vs. acetazolamide +33 (12–45, $P < 0.001$). SF-36 quality of life was similar with all treatments. Nocturnal oxygen saturation significantly improved with both NOT and acetazolamide. Right ventricular fractional area change was greater on NOT compared with placebo ($P = 0.042$) and acetazolamide ($P = 0.027$).

Conclusions In patients with pre-capillary PH and SDB on optimized pharmacological therapy, NOT improved the 6 MWD compared with placebo already after 1 week along with improvements in SDB and haemodynamics.

ClinicalTrials.gov NTC01427192.

Keywords Hypertension • Pulmonary • Pulmonary heart disease • Sleep • Hypoxia

Introduction

Pre-capillary pulmonary hypertension (PH) is a severe condition leading to progressive right heart failure with impaired quality of life, reduced exercise capacity and pre-mature death.[1] In the absence of relevant lung diseases, the two major groups are pulmonary arterial hypertension (PAH), including idiopathic and associated forms, and chronic thromboembolic pulmonary hypertension

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(CTEPH).[1] Treatment consists in prescription of drugs and, in selected cases, pulmonary endarterectomy or lung transplantation.[2] Sleep reduces respiratory drive, upper airway stability and may therefore lead to ventilatory instability with apnoea/hypopnoea associated with intermittent hypoxaemia, or sustained hypoxaemia due to a relative hypoventilation.[3] Hypoxaemia induces pulmonary vasoconstriction and may aggravate PH by its effects on pulmonary vascular smooth muscle and endothelial cells.[4] Sleep-disturbed breathing (SDB) including sleep apnoea and nocturnal hypoxaemia affect more than two-thirds of patients with right heart failure due to PH.[5–7] As in left heart failure, PH with right ventricular dysfunction might promote SDB by a delayed and enhanced chemoreflex feedback, and by an increased plant gain resulting in unstable feedback control.[8] Sleep-disturbed breathing in patients with PH is clinically relevant since it may further impair daytime performance, exercise capacity, pulmonary haemodynamics, and quality of life.[5] Sleep-disturbed breathing may also adversely affect prognosis as reported in patients with left heart failure.[5]

The indications, types and benefits of different potential treatments for SDB in pre-capillary PH are currently not known. Extrapolating from left heart failure, nocturnal oxygen therapy (NOT), acetazolamide, or non-invasive positive pressure ventilation via a mask may be effective.[9,10] In the present randomized, placebo-controlled trial, we tested the hypothesis that treatment with NOT or acetazolamide, respectively, during 1 week ameliorates exercise capacity, quality of life and SDB in patients with pre-capillary PH.

Methods

Design and setting

This is a randomized, double-blind, sham and placebo-controlled three-period cross-over trial in patients with PH and SDB. The study compared effects of (i) nocturnal supplemental oxygen by nasal cannula with a flow rate of 3 l/min (NOT) and placebo tablets, (ii) acetazolamide tablets and sham-NOT (room air by nasal cannula with a flow rate of 3 l/min), with (iii) sham-NOT and placebo tablets (*Figure 1*). In the following, these three treatment combinations are termed NOT, acetazolamide, and placebo, respectively. Each treatment was applied for 1 week in the patients' home. Assessments took place in the last night of each treatment period and in the following morning at the Pulmonary Clinic, University Hospital of Zurich. During washout periods of 1 week, patients did not receive any study treatment. The trial was performed from December 2010 to August 2012. The study was approved by the local ethics review board. The trial is registered at clinicalTrials.gov: NTC-01427192.

Patients

Consecutive patients aged 20–80 years, either gender, diagnosed with PAH or inoperable CTEPH (WHO group I or IV) at our tertiary care outpatient clinic were eligible for enrolment upon written informed consent. All patients were diagnosed according to current guidelines and had undergone right heart catheterization at the time of initial evaluation.[1] Patients were considered for inclusion if they were in a stable condition on the same medication for >4 weeks. Eligible patients had no severe daytime hypoxaemia (PaO_2 ≥7.3 kPa) but suffered from SDB defined as either a mean nocturnal oxygen saturation (SpO_2) <90% or an oxygen desaturation index (ODI, >3% dips) >10 dips/h during an ambulatory nocturnal pulse oximetry. Patients with PaO_2 <7.3 kPa during daytime, predominantly obstructive sleep apnoea, more than mild lung disease (forced expiratory volume in 1 s ≤60%) or concomitant left ventricular disease were excluded.

Interventions, randomization, and blinding

NOT (or sham-NOT corresponding to room air) was delivered via a nasal cannula at a flow rate of 3 L/min by an oxygen concentrator (Respironics EverFloTM, Zofingen, Switzerland). The sham-concentrators were prepared by modifying the same type of concentrators to provide room air instead of oxygen at identical flow rates. Patients were instructed in the use of the oxygen (or sham) concentrators by a blinded investigator who delivered the device to the patient's home and collected it at the end of each treatment phase.

A set of the study medication was given to the patient at the beginning of each study period. Acetazolamide (Diamox, Vifor, Fribourg, Switzerland) was administered at a dose of 2 × 250 mg/day with breakfast and dinner, respectively. Similarly, capsules containing acetazolamide and placebo were prepared by the Cantonal pharmacy of Zurich and packed in containers labelled with a code that was broken only after data analysis.

Allocation to one of the six possible study sequences was performed by an independent pharmacist by a computer generated randomization list assuring a balanced block design. Patients and investigators participating in evaluation of outcomes were blinded to the treatment (double-blind design).

Assessments

Assessments were performed on the last day/night of each 1-week treatment period (*Figure 1*).

A 6 min walk distance (MWD) was assessed at 10–11 a.m. after the completion of all other assessments by experienced nurses blinded to the treatment.[11] Quality of life was assessed by the 1-week-recall form SF-36 and the Minnesota living with heart failure questionnaire.[12,13] Sleepiness was assessed by the Epworth sleepiness scale.[14] Medical history, NYHA/WHO functional class, and physical examination were assessed.

Patients were examined by echocardiography at 9 a.m. after the sleep study. Cardiac morphological and functional parameters were assessed by standard two-dimensional Doppler echocardiography (Philips iE33; Philips, Zofingen, Switzerland) by an experienced cardiologist blinded to the patients' clinical data and treatment (Felix C. Tanner). The dimensions of right ventricle and right atrium, right ventricular fractional area change, and tricuspid annular plain systolic excursion were determined.[15,16] Maximal systolic flow velocity of the tricuspid regurgitation jet was measured by continuous wave Doppler and the maximal systolic pressure gradient between right ventricle and right atrium calculated using the simplified Bernoulli equation; right atrial pressure was estimated by the dimension and respiratory variability of the inferior cava vein.[15,16]

A radial artery blood sample was obtained while the patient was resting quietly breathing room air. Analysis was performed immediately (ABL 90Flex-blood gas analyser, Radiometer, Switzerland). NT-pro brain natriuretic peptide was analysed in a venous blood sample by immunoassay (Roche Modular Systems, Rotkreuz, Switzerland).

Polysomnography was performed from ~10 p.m. to 6:30 a.m. in the sleep laboratory according to standard methods in the last night of each treatment period.[5,17,18] Measurements included electroencephalography, electrooculography, electromyography of submental and tibial muscles, nasal pressure to assess airflow, oral thermistor, calibrated respiratory inductance plethysmography, pulse oximetry, transcutaneous carbon dioxide tension, electrocardiogram, body position, and audio-visual recordings (Alice5, Philips, Respironics, USA).[5] Sleep studies were analysed as described previously.[5] An apnoea/hypopnoea was defined as a reduction of the breathing amplitude to <50% in comparison to the preceding baseline lasting for ≥10 s. Central apnoea/

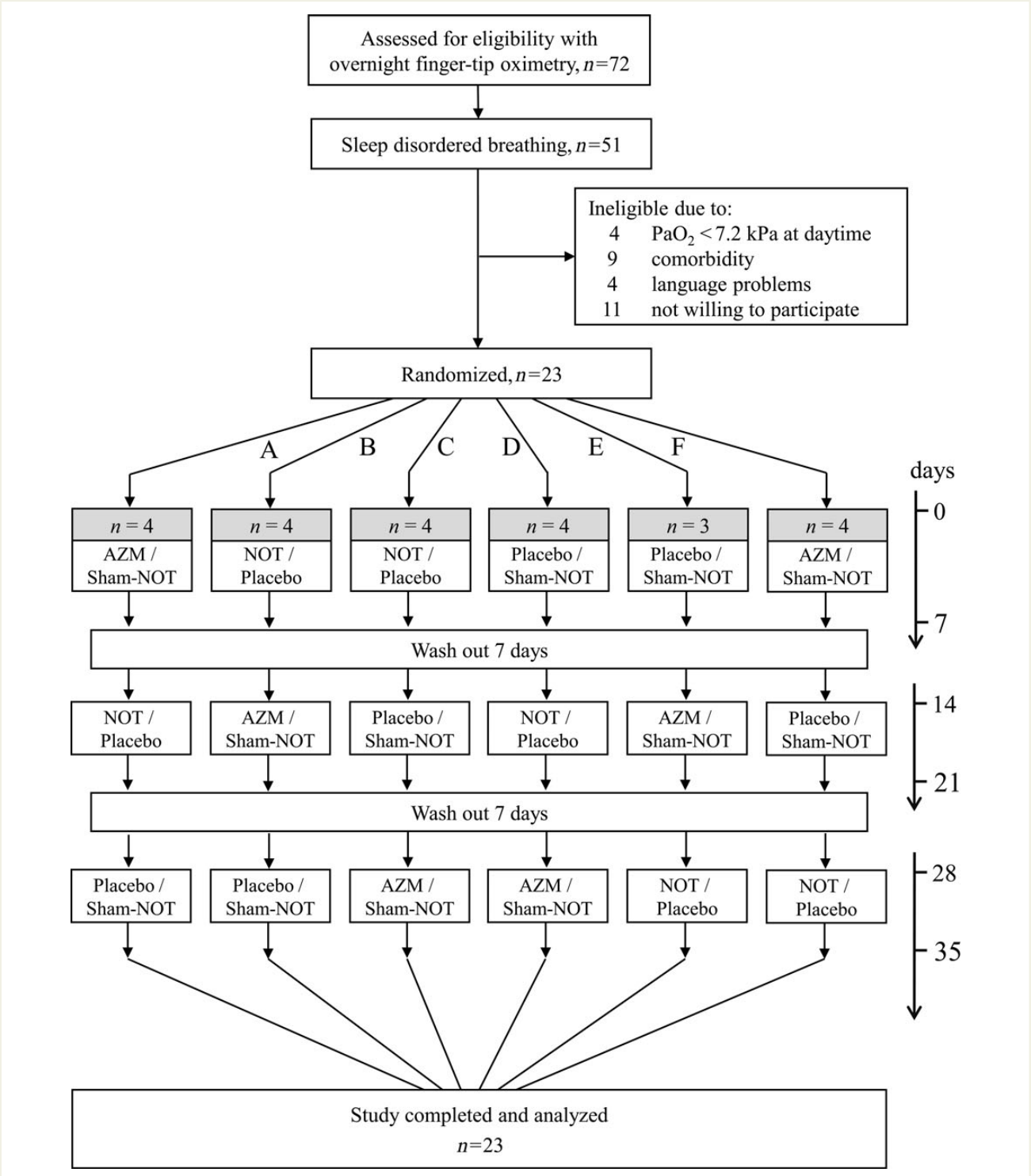

Figure 1 Patient flow. PaO_2, partial pressure of arterial oxygen; AZM, treatment with acetazolamide tablets 250 mg twice a day; NOT, nocturnal oxygen therapy via a nasal cannula at a flow rate of 3 L/min. Sham-NOT, room air at a flow rate of 3 L/min. Placebo, tablets twice a day.

hypopnoeas were differentiated from obstructive events by the absence of asynchronous and paradoxical rib cage-abdominal excursions and by diaphragmatic surface EMG. The apnoea/hypopnoea index (AHI) was computed as the number of events per hour. Periodic breathing was scored when at least three continuous cycles of waxing and waning tidal volumes were present with periods of hyperventilation separated by central apnoeas or hypopnoeas.

Psychomotor vigilance tests (PVTs) were performed in a quiet room between 7 and 8 a.m. The reaction time to a light signal appearing at irregular intervals was measured during a 15 min session.[19]

Outcomes

Primary outcomes were the 6MWD and the SF-36 physical component summary. Additional outcomes were the SF-36 and Minnesota living with heart failure quality-of-life domains, WHO/NYHA functional class, echocardiographic assessments of the tricuspid pressure gradient, the tricuspid annular plane systolic excursion and right ventricular fractional area change, arterial blood gases, venous N-terminal brain natriuretic peptide, and variables derived from sleep studies such as nocturnal oxygen saturation, sleep apnoea events, sleep structure, and PVT reaction time.

Data analysis and statistics

We calculated that a sample size of 22 patients was required including a 10% drop-out rate to detect minimally important differences in primary outcomes (35 m for the 6 min walk and 9 points for the SF-36 physical scale) with 80% power and a two-sided significance level of <0.05.[20–22] Results are reported as number (%), medians (quartiles), and mean differences with 95% CI. All analyses were performed on an intention-to-treat basis. Missing data were replaced by the corresponding value on the alternative intervention or the group mean conservatively assuming no effect of the respective treatment.[23] Outcomes were analysed by analysis of variance with Bonferroni correction and Wilcoxon matched pairs tests. Repeated measures analysis of variance was performed to assess potential effects of exposure sequence and time effects. Statistical significance was assumed at $P < 0.05$.

Results

Of 72 PH-patients screened, 23 met the inclusion criteria and were randomized (*Figure 1*). Twenty-eight patients could not be included, although they had sleep-related hypoxaemia, because they could not undergo assessment of the main outcome, the 6MWT ($n = 9$), had daytime hypoxaemia (PaO_2 <7.3 kPa) ($n = 4$), or did not consent. Baseline characteristics of these 28 patients were similar to the 23 patients participating in the trial (Supplementary material online, *Table S1*). Patients were predominantly females with idiopathic PAH or inoperable CTEPH. According to entry criteria, patients were in stable condition, WHO/NYHA functional classes II to IV, on optimized single or multiple PH target therapies (*Table 1*). All participants completed the study. The 6MWD could not be obtained in one patient after NOT and acetazolamide who was unable to walk for 6 min due to arthritic hip pain, another patient did not fill the SF-36 questionnaire during the study period on acetazolamide.

Table 1 Baseline characteristics

Characteristic	Number (%) or median (quartiles)
Number of participants (females)	23 (15)
Age (year)	66 (56; 71)
BMI (kg/m^2)	26.6 (25.2; 29.3)
NYHA class (II, III, IV)	6 (26), 14 (61), 3 (13)
Classification, *n* (%)	
Pulmonary arterial hypertension	16 (70)
Idiopathic	13 (57)
Connective tissue disease	1 (4)
Congenital heart disease	1 (4)
Porto-pulmonary	1 (4)
Chronic thromboembolic PH	7 (30)
Mean pulmonary arterial pressure (mmHg)[a]	44 (32;52)
Pulmonary capillary occlusion pressure (mmHg)[a]	11 (9;13)
Pulmonary vascular resistance (dyn s m^{-5})[a]	505 (373;742)
6MWD (m)	448 (375;501)
Daytime oxygen saturation (%)	93 (91;95)
Mean nocturnal oxygen saturation (%)	87 (86;89)
Oxygen desaturation index (>3%) (1/h)	14 (4;24)
PH-specific medication	
PDE-5 inhibitor	11 (48)
Endothelin receptor antagonist	18 (78)
Prostanoid therapy	4 (17)
Patient on combination therapy	
Two-drug combination	6 (26)
Three-drug combination	4 (17)

Data obtained during screening or [a]obtained during last right heart catheterization.

Primary outcomes

Exercise capacity and quality of life

After 1 week of NOT, the 6MWD was significantly greater than the 6MWD on placebo, respectively, acetazolamide (*Table 2*, *Figure 2*). Acetazolamide had no significant effect on the 6MWD compared with placebo. Quality of life assessed by the 1 week-recall form of SF-36 was similar on all treatments (*Table 2* and Supplementary material online, *Table S2*).

Additional outcomes

Functional class, disease-specific quality of life and sleepiness

WHO/NYHA functional classes I/II/III/IV were found in 0/7/12/4 patients after placebo, 1/11/8/3 after NOT and 0/7/11/5 after acetazolamide (*Figure 3*). Thus, five more patients were in the target WHO/NYHA classes I/II with NOT compared with placebo and acetazolamide (absolute risk reduction 0.21, number needed to treat 5). The Minnesota living with heart failure questionnaire and the Epworth sleepiness scale revealed no difference between treatments (*Table 2* and Supplementary material online, *Table S1*).

Echocardiography

We found a significantly higher right ventricular fractional area change with NOT compared with placebo (*Table 2*). Tricuspid annular plane systolic excursion and the tricuspid valve systolic pressure gradient were comparable in all groups.

Sleep and vigilance studies

Nocturnal oxygen therapy and acetazolamide both significantly improved the total and central AHI, the percentage of the night spent with periodic breathing and the mean nocturnal oxygen saturation compared with sham-NOT/placebo (*Table 2*). In addition, NOT increased the amount of deep sleep (NREM stages III and IV) which

Table 2 **Effects of nocturnal oxygen and acetazolamide treatment**

	Placebo/ sham-O_2	NOT	Acetazolamide	ΔNOT − placebo (95% CI)	*P* Wilcoxon	Δacetazolamide − placebo (95% CI)	*P* Wilcoxon	ΔNOT − aceta-zolamide (95% CI)	*P* Wilcoxon	*P* ANOVA (overall)
Exercise capacity										
6MWD	454 (366, 510)	480 (390, 530)	440 (366, 472)	25 (3 to 46)	0.027	−9 (−34 to 17)	0.223	33 (21 to 46)	<0.001	<0.001
Quality of life										
SF-36 physical component summary	38 (34, 44)	41 (33, 47)	38 (33, 49)	2 (−1 to 4)	0.140	−1 (−4 to 2)	0.974	3 (−1 to 7)	0.263	0.293
SF-36 mental component summary	58 (53, 62)	58 (53, 62)	59 (47, 62)	1 (−3 to 4)	0.695	−4 (−7 to −0)	0.052	4 (−1 to 9)	0.211	0.153
WHO/NYHA functional class	3 (2;3)	2 (2;3)	3 (2;3)	0 (−1 to 0)	0.052	0 (0 to 0)	0.705	0 (−1 to 0)	0.021	0.037
MLHF general	17.0 (13.0, 37)	20.0 (9.5, 47.0)	17.0 (7.0, 30.0)	1 (−3 to 5)	0.438	−2 (−6 to 2)	0.306	4 (−1. to 8)	0.163	0.444
Echocardiography										
Right ventricular fractional area change (%)	32 (19; 35)	32 (21; 38)	30 (18; 35)	2 (0 to 4)	0.042	0 (−2 to 2)	0.984	2.0 (0.1 to 4.0)	0.027	0.022
Tricuspid pressure gradient (mmHg)	62 (43; 86)	56 (44; 71)	53 (47; 75)	−2.5 (−7 to 2)	0.420	−4 (−8 to 0)	0.068	2 (−2 to 5)	0.449	0.412
Tricuspid annular plane systolic excursion (mm)	21.0 (18.0; 23.0)	20.5 (18.0; 23.0)	20.5 (18.0; 23.0)	0.2 (−1 to 1)	0.653	0 (−1 to 1)	0.796	0.2 (−1.2 to 1.7)	0.925	0.905
Sleep studies and vigilance										
Mean nocturnal arterial oxygen saturation (%)	86 (84, 89)	92 (91, 94)	90 (88, 92)	6 (4 to 7)	<0.001	3 (2 to 4)	<0.001	2 (1 to 4)	0.001	<0.001
Oxygen desaturation index (ODI)	7 (0, 16)	0 (0, 3)	2 (1, 13)	−11 (−20 to −2)	0.002	−4 (−10 to 1)	0.105	−7 (−13 to 0)	0.021	0.006
Total AHI (1/h)	18 (6, 40)	9 (6, 24)	7 (3, 27)	−11 (−17 to −3)	0.002	−10 (−18 to −1)	0.004	0 (−7 to 6)	0.976	<0.001
Periodic breathing (% total sleep time)	8 (2, 22)	1.8 (0, 7)	2.6 (0, 14)	−12 (−20 to −4)	<0.001	−8 (−15 to −1)	0.030	−4 (−11 to 3)	0.309	<0.001
Mean reciprocal reaction time (response speed, 1/s)	3.5 (3.2, 3.9)	3.7 (3.5, 3.9)	3.3 (3.1, 3.49)	0.2 (−0.2 to 0.5)	0.543	−0.2 (−0.5 to −0.0)	0.004	0.4 (0.2 to 0.7)	<0.001	<0.001
Blood analysis										
Arterial pH	7.43 (7.41, 7.45)	7.43 (7.40, 7.45)	7.35 (7.33, 7.37)	0 (0 to 0)	0.073	−0.1 (−0.1 to −0.1)	<0.001	0.1 (0.06 to 0.09)	<0.001	<0.001
PaO_2 (kPa)	7.8 (6.9, 8.5)	7.5 (6.9, 8.2)	8.7 (8.1, 9.5)	−0.1 (−0.9 to 0.7)	0.465	0.9 (0.3 to 1.5)	0.004	−1.0 (−1.6 to −0.3)	0.008	<0.001
$PaCO_2$ (kPa)	4.4 (4.1, 4.9)	4.6 (4.3, 5.1)	3.9 (3.5, 4.2)	0.2 (0.1 to 0.4)	0.010	−0.6 (−0.7 to −0.5)	<0.001	0.8 (0.7 to 0.9)	<0.001	<0.001

Continued

Table 2 *Continued*

	Placebo/ sham-O_2	NOT	Acetazolamide	ΔNOT− placebo (95% CI)	*P* Wilcoxon	Δacetazolamide− placebo (95% CI)	*P* Wilcoxon	ΔNOT − aceta-zolamide (95% CI)	*P* Wilcoxon	*P* ANOVA (overall)
Bicarbonate (mmol)	22.0 (20.3, 23.5)	22.5 (20.7, 23.8)	16.6 (14.6, 17.3)	0.3 (−0.4 to 1.1)	0.280	−5.9 (−6.7 to −5.1)	<0.001	6.2 (5.5 to 6.8)	<0.001	<0.001
NT-pro-brain-natriuretic peptide (mmol/L)	348 (75, 1208)	289 (124, 968)	178 (90, 880)	−125 (−424 to 174)	0.224	−155 (−352 to 43)	0.026	29 (−138 to 197)	0.927	0.086

Data are medians (quartiles) and between-treatment differences are means (95% CI). *P* by Wilcoxon matched pairs test for comparisons between treatments and repeated-measurement ANOVA for all differences. The SF-36 quality-of-life questionnaire physical and mental component summaries range from 0 (poorest) to 100 (best and health).
MLHF, Minnesota living with heart failure questionnaire; PaO_2, partial pressure of arterial oxygen in kPa (*7.5 gives mmHg); $PaCO_2$, partial pressure of arterial carbon dioxide in kPa (kPA*7.5 gives mmHg); Δ, difference between NOT, respectively, acetazolamide with sham-NOT/placebo.

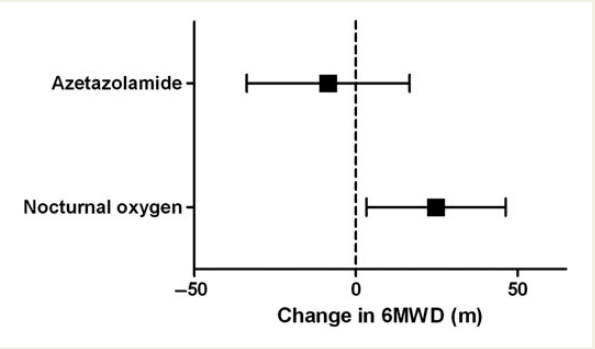

Figure 2 Change in 6 min walk distance after nocturnal oxygen therapy and acetazolamide. The mean differences with the corresponding 95% confidence interval in the 6 min walk distance after 1 week acetazolamide tablets, respectively, nocturnal oxygen therapy compared with placebo/sham-oxygen are shown.

was not observed with acetazolamide (Supplementary material online, *Table S3*). Psychomotor vigilance test reaction speed was reduced on acetazolamide compared with the other treatments. Additional results of sleep studies and vigilance tests are shown online (Supplementary material online, *Table S3*).

Arterial and venous blood analyses

The arterial pH was significantly reduced with acetazolamide compared with placebo ($P < 0.001$) along with an increased PaO_2, decreased $PaCO_2$, and bicarbonate (*Table 2*). NT-pro-BNP was lower after acetazolamide compared with placebo ($P = 0.007$).

Tolerability, drug accountability, preferences, and influence of treatment sequences

Nocturnal oxygen therapy and acetazolamide were subjectively well tolerated with no major adverse events reported by the patients. Prickling sensation in the fingers, dry nose, and fatigue were reported by 1/3/0 after sham-NOT/placebo, 0/3/0 after NOT and 3/4/3 after acetazolamide. Drug accountability after each 1 week treatment period indicated that all patients took the tablets as prescribed. After NOT, placebo, and acetazolamide 19, 19, and 20/23 patients (83, 83, and 87%) reported that they would use NOT if it would help and only one patient declared not to use NOT regardless of any benefit. However, 20, 20, and 21/23 patients (87 resp. 91%) would prefer tablets to NOT therapy if it would help equally. Three, 1, and 0 patients each had no preferences on NOT, placebo, and acetazolamide each. To evaluate the effect of treatment sequence on the 6MWD analysis of variance was performed with treatment sequence and type of treatment as independent variables. The results confirmed a significant effect of treatment ($P = 0.004$) but did not suggest any effect of the treatment sequence ($P = 0.157$, P interaction $= 0.364$).

An exploratory subgroup analysis revealed that patients with PAH ($n = 16$) tended to improved their 6MWD during NOT compared with placebo (481 (406;557) vs. 460 (386;533) m, $P = 0.076$) and significantly improved it compared with acetazolamide (440 (372;507) m, $P = 0.004$). The seven patients with CTEPH tended to improve

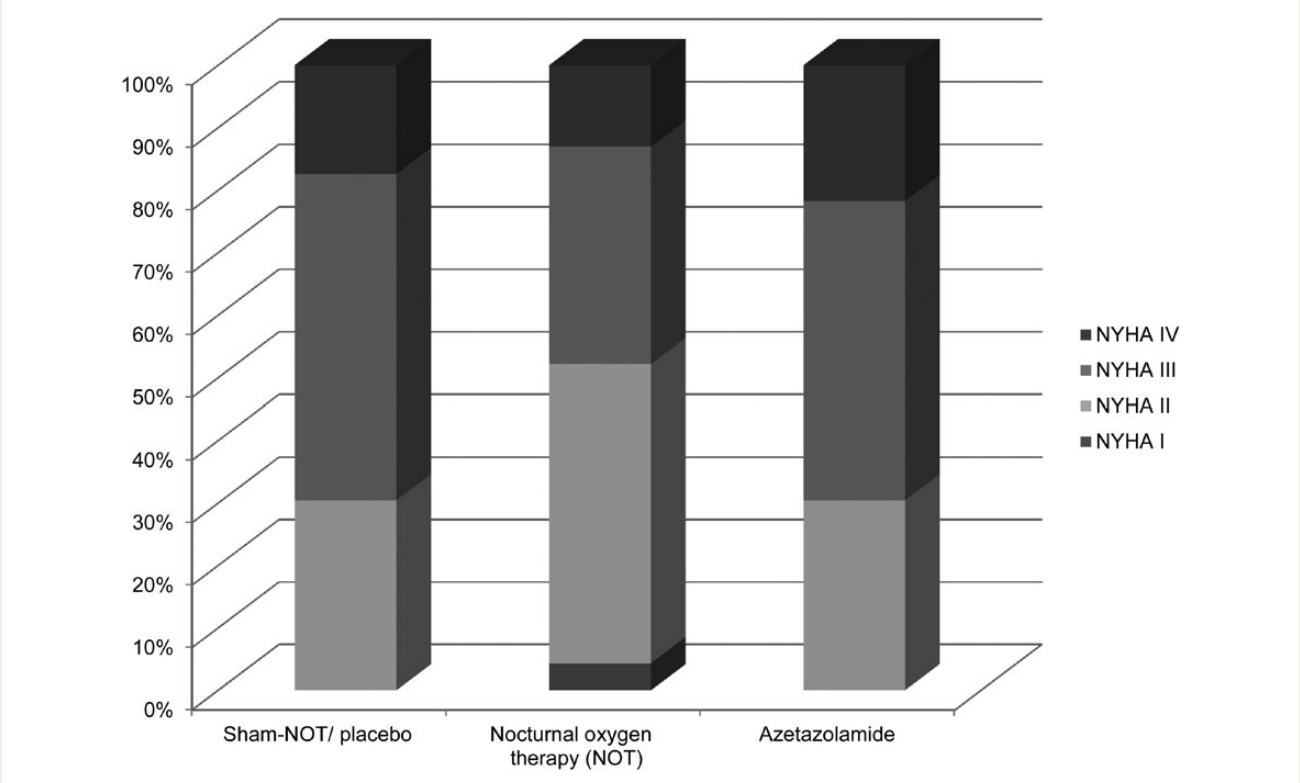

Figure 3 Distribution of WHO/NYHA functional classes after each treatment period. The percentage of patients in WHO/NYHA functional classes' I–IV is shown as assessed after each 1 week treatment period: nocturnal oxygen therapy, acetazolamide, and sham-nocturnal oxygen therapy/placebo, respectively.

their 6MWD during NOT (444 (356;532) vs. 426 (319;533) m, $P = 0.173$ and significantly improved it vs. acetazolamide (432 (350;314), $P = 0.028$). The differences in quality-of-life scales were not different for the pulmonary arterial and CTEPH (data not shown).

Discussion

This randomized, sham/placebo-controlled, double-blind trial in patients with PAH and CTEPH suffering from SDB demonstrates that 1 week of NOT improved the 6MWD compared with placebo and acetazolamide, respectively. The favourable effect of NOT on exercise performance was associated with improvements in right ventricular fractional area change, in sleep-related breathing disturbances and with a reduced WHO/NYHA functional class in 8 of 23 patients (35%). In contrast, treatment with acetazolamide did not change exercise performance, functional class and pulmonary haemodynamics, although SDB was improved to a similar degree as with NOT.

Sleep induces profound physiological alterations to the respiratory system even in healthy subjects.[24] Possible underlying mechanisms are mismatched ventilation-perfusion, reduced functional residual capacity due to recumbent position and reduced respiratory drive. Pre-existing cardiopulmonary diseases potentiate these mechanisms and worsen SDB.[25] Thus, periodic breathing and nocturnal hypoxaemia are highly prevalent in patients with left heart failure, lung diseases, and pre-capillary PH[5–7,26,27] and SDB is underestimated by daytime office assessments.[6] In analogy to patients with left heart failure,[9,10] we tested whether NOT or acetazolamide would improve SDB and herewith exercise performance, symptoms, quality of life and pulmonary haemodynamics in PH. Both, NOT and acetazolamide, improved SDB in PH, but only NOT improved the 6MWD in comparison to placebo (by a mean of 25). This improvement is remarkable for several reasons: NOT improved the 6MWD even in patients on optimized pharmacological PH target therapy (44% of the patients were even on double or triple PH target therapy). Several randomized-controlled studies investigating the effect of target medication for pre-capillary PH over 12–16 weeks used the 6MWD as endpoint. The gain in 6MWD in these studies was mostly comparable with our study.[28,29] In contrast to our study, a significant increase in the 6MWD was often not achieved in randomized-controlled trials by adding drugs to patients already on PH target therapy (combination therapy).[30–33] Moreover, in the present trial, NOT was given for a short period of 1 week only and the 6MWD was performed ~10–11 a.m., ~4 h after cessation of NOT. In analogy to our study, a 1 week therapy with NOT-improved SDB and exercise capacity in patients with left heart failure.[34] Thus, the improvement on NOT in patients with left- and right heart failure and SDB seems to be comparable. The fact that acetazolamide did not increase the 6MWD, although it improved sleep-related breathing disturbances to a similar degree as NOT suggests that the two treatments may act via different physiologic pathways as will be further discussed below.

In our short-term study, we did not find a significant difference in quality of life assessed by the 1-week-recall form of the SF-36 physical component score, SF-36 domain scores or quality of life by the Minnesota living with heart failure questionnaire, a disease-specific instrument. Potentially, the duration of our study was too short to consistently ameliorate quality of life. Nevertheless, NOT had a favourable effect on symptoms, as one-third of patients improved their WHO/NYHA functional class on NOT (*Figure 3*) resulting in additional five patients reaching the treatment goal of being in Class I/II after 1 week of NOT (totally 12 patients compared with 7 on sham-NOT or acetazolamide). Thus, five patients would need treatment for 1 week in order to have one patient in the target WHO/NYHA class II. These results underscore that NOT, albeit given for a short time only, has the potential to improve patient centred outcomes.

Right heart function is a main determinant of disease severity and outcome in pre-capillary PH.[35–37] NOT therapy was associated with a significant improvement in right ventricular function reflected in a higher fractional area change. Supplemental oxygen has been shown to improve right ventricular function in patients with chronic obstructive pulmonary disease in association with a decreased pulmonary vascular resistance.[38,39] However, our study failed to show an improvement of the tricuspid valve systolic pressure gradient, as surrogate of pulmonary artery pressure. Possibly, the treatment period of 1 week was too short to improve several markers of right ventricular function and our study may have been underpowered to detect minor changes in some of the secondary outcomes. Nevertheless, the observed increase in right ventricular fractional area change points towards a right heart functional improvement by NOT and warrants further study.

We found that acetazolamide and NOT were highly effective in improving SDB with a significant decrease in central sleep apnoea, reduction in periodic breathing, oxygen desaturation index, and a marked improvement in nocturnal SpO_2. This corresponds with improvements in SDB previously described for patients with left heart failure on these therapies or the improvement of periodic breathing in mountaineers or patients with obstructive sleep apnoea travelling to altitude.[9,34,40–42] Acetazolamide has been shown to improve hypoxic pulmonary vasoconstriction in dogs,[43] to ameliorate pulmonary haemodynamic in hypoxia-exposed rats and its prophylactic effect for high-altitude-associated illness is well known.[44] In contrast to NOT, acetazolamide did not ameliorate exercise capacity or function in our study. This might be partly explained by the introduction of metabolic acidosis by acetazolamide, which had to be compensated by increased ventilation in PH-patients which already tend to hyperventilate. Arterial blood gases of our patients on acetazolamide revealed an increased partial pressure of oxygen along with a decreased partial pressure of carbon dioxide, bicarbonate, and pH. Acidosis may also impair pulmonary haemodynamics by an increased pulmonary vascular constriction. On the other hand, the diuretic effect of acetazolamide might have decreased right atrial pressure, right ventricular preload and thereby might have lowered the tricuspid valve systolic gradient, a tendency which was found in our cohort (*Table 2*, $P = 0.068$). Despite higher partial pressures of oxygen due to hyperventilation, acetazolamide is associated with a less efficient breathing and respiratory muscle fatigue,[45,46] which might further explain the impaired exercise performance in our PH-cohort during acetazolamide therapy. However, it may well be that acetazolamide given only before sleep and not in the morning, would have improved exercise capacity during the day as well as improved SDB.

Although we could not include several screened patients with nocturnal hypoxaemia into the current trial because they could not undergo assessment of the main outcome, the 6MWT, and for other reasons (*Figure 1*) we feel that the proportion of PH patients who might benefit from NOT is relatively large since 51 of 72 consecutive patients with pre-capillary PH, i.e. 79% revealed nocturnal hypoxaemia that might respond favourably to NOT.

Our study has important clinical implications: patients with pre-capillary PH who suffer from periodic breathing and/or nocturnal hypoxaemia, may benefit from NOT even if daytime arterial oxygenation is relatively well preserved. Compared with pharmacological pH therapies, NOT is an inexpensive therapy without serious side effects.

In summary, this randomized, placebo-controlled, double-blind trial demonstrates for the first time that treatment with NOT not only ameliorates nocturnal oxygenation and periodic breathing in patients with PAH or inoperable CTEPH and SDB but also improves exercise capacity, functional class and potentially right ventricular function already within 1 week. As NOT is inexpensive and save, SDB should be diagnosed and NOT considered in these patients. The long-term effect of NOT in PH-patients with SDB and preserved daytime oxygenation should be studied in future randomized trials.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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